

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference FSU-100C2XC1	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/US 00/ 27900	International filing date (day/month/year) 06/10/2000	(Earliest) Priority Date (day/month/year) 06/10/1999
Applicant FLORIDA STATE UNIVERSITY RESEARCH FOUNDATION		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

12

Applicant's or agent's file reference REP06739EP	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US00/27900	International filing date (day/month/year) 06/10/2000	Priority date (day/month/year) 06/10/1999
International Patent Classification (IPC) or national classification and IPC C12N15/00		
Applicant FLORIDA STATE UNIVERSITY RESEARCH FOUNDATION et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 5 sheets, including this cover sheet.

- ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 04/05/2001	Date of completion of this report 09.01.2002
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4455	Authorized officer Trommsdorff, M Telephone No. +49 89 2399 7361 

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/27900

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):
Description, pages:

1-28 as originally filed

Claims, No.:

1-59 as amended under Article 19

Drawings, sheets:

2/2 as originally filed

1/2 as filed with the letter of 07.11.01

Sequence listing part of the description, pages:

1-13, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/27900

listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-59
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-59
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-57
	No:	Claims	58, 59: no opinion

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1. Cited documents

The following documents (D) are referred to in this communication; the numbering is the same as in the search report and will be adhered to in the rest of the procedure:

- D1: THOREAU V ET AL: 'Molecular cloning, expression analysis, and chromosomal localization of human Syntaxin 8 (STX8)' BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 257, 1999, p.577-583, ISSN: 0006-291X
- D2: KUNZELMANN K ET AL: 'Inhibition of epithelial Na⁺ currents by intracellular domains of the cystic fibrosis transmembrane conductance regulator.' FEBS LETTERS, vol. 400, no. 3, 1997, p.341-4 ISSN: 0014-5793
- D3: NEVILLE DAVID C A ET AL: 'Expression and characterization of the NBD1-R domain region of CFTR: Evidence for subunit-subunit interactions.' BIOCHEMISTRY, vol. 37, no. 8, 24 February 1998 (1998-02-24), p.2401-9, ISSN: 0006-2960
- D5: WO 94 25607 A (UNIV IOWA RES FOUND) 10 November 1994 (1994-11-10)

2. Amendments

The amendments filed with the International Bureau under Article 19(1) do not introduce subject-matter which extends beyond the content of the application as filed and thus comply with Article 19(2) PCT.

3. Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

- 3.1. The claims are directed to a method for determining the interaction of CFTR polypeptides using, e.g. the two-hybrid-system and the corresponding host cells. Several prior art documents use the two-hybrid-system to test interactions between CFTR and other proteins. D1, for example, shows that syntaxin 8 interacts with the regulator domain (R) of CFTR. D2 also tests different domains of CFTR in the two-hybrid-system. The results suggest an interaction between the cytoplasmic domain of CFTR and the alpha subunit of the amiloride sensitive Na⁺ channel (ENAC) (p.342, table 1). D5 describes a method to test compounds which

restore CFTR function using yeast STE6-CFTR chimeric sequences.

Other prior art documents use different methods to analyse protein-protein interaction:

D3 shows that the nucleotide binding domain 1 (NBD1) of CFTR affects phosphorylation of the regulatory domain of CFTR probably by inducing a conformational change of the protein.

However, none of the available prior art documents discloses or even suggests an interaction between two CFTR domains which could lead to a dimerization of the CFTR protein.

Hence, the subject-matter of claims 1-59 is novel and inventive (Art. 33(1)-(3) PCT).

- 3.2. Claims 58 and 59 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

4. Re Item VIII

Certain observations on the international application

- 4.1. Claim 58 is not supported by the description as required by Article 6 PCT, as its scope is broader than justified by the description and drawings. The reasons therefor are the following: in the examples, the applicants show an interaction between the two NBD1 domains of CFTR and suggest that the dimerization of NBD1 is required for processing of CFTR. Since however other mechanisms not discussed by the applicants could also be involved in CFTR processing (see Denning et al., Nature 358, p.761-4), the claim should be restricted to an improvement of CFTR processing as a consequence of dimerization.

Claims

I claim:

- 1 1. A method for detecting or determining the interaction of a first CFTR
2 polypeptide with a second CFTR polypeptide, said method comprising:
3 (a) providing a first polynucleotide encoding a fusion protein comprising all or a
4 portion of a first CFTR polypeptide and a DNA binding domain of a transcriptional
5 activator that can bind to a site on a detectable gene;
6 (b) providing a second polynucleotide encoding a fusion protein comprising all or
7 a portion of a second CFTR polypeptide and a transcriptional activation domain of a
8 transcriptional activator that can activate transcription of said detectable gene;
9 (c) incorporating said first and second polynucleotide into a host cell comprising
10 said detectable gene wherein transcription of said detectable gene is under control of said
11 transcriptional activator;
12 (d) expressing said polynucleotide encoding said first CFTR polypeptide and said
13 second CFTR polypeptide under conditions in which said detectable gene is expressed
14 when said first CFTR polypeptide and said second CFTR polypeptide interact; and
15 (e) detecting transcription of said detectable marker gene or expression of the gene
16 product of said detectable gene.
- 1 2. The method according to claim 1, wherein said host cell is a yeast cell.
- 1 3. The method according to claim 2, wherein said yeast cell is *Saccharomyces*.
- 1 4. The method according to claim 1, wherein the host cell is a mammalian cell.
- 1 5. The method according to claim 1, wherein said CFTR polypeptide is a
2 mammalian CFTR polypeptide.
- 1 6. The method according to claim 1, wherein said CFTR polypeptide comprises
2 amino acid residue 351 through 650 of the human CFTR protein sequence.

1 7. The method according to claim 1, wherein said detectable gene is selected from
2 the group consisting of *lacZ*, *LEU2* and *HIS3*.

1 8. The method according to claim 1, wherein said DNA binding domain comprises
2 the DNA binding domain of GAL4 protein.

1 9. The method according to claim 1, wherein said transcriptional activation domain
2 comprises the transcriptional activation domain of GAL4 protein.

1 10. The method according to claim 1, wherein said CFTR polypeptides are mutant
2 CFTR polypeptides.

1 11. The method according to claim 1, wherein said CFTR polypeptide comprises
2 a mutation in the first nucleotide binding domain (NBD1).

1 12. The method according to claim 10, wherein said mutant CFTR polypeptide
2 contains a $\Delta F508$ mutation.

1 13. The method according to claim 1, wherein said CFTR polypeptide is a wild
2 type CFTR polypeptide.

1 14. A method of identifying a compound that facilitates interaction of CFTR
2 polypeptides, said method comprising:

3 (a) contacting a host cell with said compound, wherein said host cell comprises
4 a polynucleotide encoding a fusion protein comprising all or a portion of a first CFTR
5 protein and a DNA binding domain of a transcriptional activator that can bind to a site on
6 a detectable gene, and a polynucleotide encoding a fusion protein comprising all or a
7 portion of a second CFTR polypeptide and a transcriptional activation domain of a
8 transcriptional activator that can activate transcription of a detectable gene, wherein said
9 host cell further comprises said detectable gene wherein transcription of said detectable
10 gene is under control of said transcriptional activator,

11 wherein said first and second CFTR polypeptides comprise a mutation that reduces or
12 prevents interaction of said fusion proteins;

13 (b) expressing said polynucleotide encoding said first CFTR polypeptide and said
14 second CFTR polypeptide under conditions in which said detectable gene is expressed
15 when said first CFTR polypeptide and said second CFTR polypeptide interact; and,

16 (c) determining whether said detectable gene is expressed in said host cell at a
17 level greater than the level of expression observed in said host cell in the absence of said
18 compound.

1 15. The method according to claim 14, wherein said host cell is a yeast cell.

1 16. The method according to claim 15, wherein said yeast cell is *Saccharomyces*.

1 17. The method according to claim 14, wherein the host cell is a mammalian cell.

1 18. The method according to claim 14, wherein said CFTR polypeptide is a
2 mammalian CFTR polypeptide.

1 19. The method according to claim 14, wherein said CFTR polypeptide comprises
2 amino acid residue 351 through 650 of the human CFTR protein sequence.

1 20. The method according to claim 14, wherein said detectable gene is selected
2 from the group consisting of lacZ, *LEU2* and *HIS3*.

1 21. The method according to claim 14, wherein said DNA binding domain
2 comprises the DNA binding domain of GAL4 protein.

1 22. The method according to claim 14, wherein said transcriptional activation
2 domain comprises the transcriptional activation domain of GAL4 protein.

1 23. The method according to claim 14, wherein said CFTR polypeptides are
2 mutant CFTR polypeptides.

1 24. The method according to claim 14, wherein said CFTR polypeptide comprises
2 a mutation in the first nucleotide binding domain (NBD1).

1 25. The method according to claim 23, wherein said mutant CFTR polypeptide
2 contains a $\Delta F508$ mutation.

1 26. The method according to claim 14, wherein said compound is present in a
2 plant and said host cells is contacted with a tissue sample from said plant.

1 27. The method according to claim 26, wherein said tissue sample is a leaf disc
2 from said plant.

1 28. The method according to claim 14, wherein said host cell is contacted with
2 a sample present or absorbed on a filter paper disc.

1 29. The method according to claim 14, wherein increased growth of said host
2 cells is used for determining whether said detectable gene is expressed in said host cells
3 at a level greater than the level of expression observed in said host cells in the absence of
4 said compound.

1 30. The method according to claim 14, wherein said CFTR polypeptide is a wild
2 type CFTR polypeptide.

1 31. The method according to claim 14, wherein said compound is selected from
2 the group consisting of a polypeptide or a biologically active fragment thereof, an
3 antibody or antigen binding fragment thereof, and a polynucleotide.

1 32. A method for detecting or determining the interaction of a first CFTR
2 polypeptide with a second CFTR polypeptide, said method comprising (a) providing a
3 fusion protein comprising all or a portion of a first CFTR protein and a DNA binding
4 domain of a transcriptional activator that can bind to a site on a detectable marker gene;
5 (b) providing a second fusion protein comprising all or a portion of a second CFTR
6 polypeptide and a transcriptional activation domain of a transcriptional activator that can
7 activate transcription of the detectable marker gene; (c) contacting said first fusion protein
8 and said second fusion protein under conditions where if said first fusion protein and said
9 second fusion protein interact then said interaction causes said transcriptional activation
10 domain to activate transcription of said detectable marker gene; and (d) detecting
11 transcription of said detectable marker gene or expression of the gene product of said
12 detectable marker gene.

1 33. The method according to claim 32, wherein said host cell is a yeast cell.

1 34. The method according to claim 33, wherein said yeast cell is *Saccharomyces*.

1 35. The method according to claim 32, wherein the host cell is a mammalian cell.

1 36. The method according to claim 32, wherein said CFTR polypeptide is a
2 mammalian CFTR polypeptide.

1 37. The method according to claim 32, wherein said CFTR polypeptide comprises
2 amino acid residue 351 through 650 of the human CFTR protein sequence.

1 38. The method according to claim 32, wherein said detectable gene is selected
2 from the group consisting of *lacZ*, *LEU2* and *HIS3*.

1 39. The method according to claim 32, wherein said DNA binding domain
2 comprises the DNA binding domain of GAL4 protein.

1 40. The method according to claim 32, wherein said transcriptional activation
2 domain comprises the transcriptional activation domain of GAL4 protein.

1 41. The method according to claim 32, wherein said CFTR polypeptides are
2 mutant CFTR polypeptides.

1 42. The method according to claim 32, wherein said CFTR polypeptide comprises
2 a mutation in the first nucleotide binding domain (NBD1).

1 43. The method according to claim 41, wherein said mutant CFTR polypeptide
2 contains a $\Delta F508$ mutation.

1 44. The method according to claim 32, wherein said CFTR polypeptide is a wild
2 type CFTR polypeptide.

1 45. A host cell comprising a polynucleotide encoding a fusion protein comprising
2 all or a portion of a first CFTR protein and a DNA binding domain of a transcriptional
3 activator that can bind to a site on a detectable gene and a polynucleotide encoding a
4 fusion protein comprising all or a portion of a second CFTR protein and a transcriptional
5 activation domain of a transcriptional activator that can activate transcription of said
6 detectable gene.

1 46. The method according to claim 45, wherein said host cell is a yeast cell.

1 47. The method according to claim 46, wherein said yeast cell is *Saccharomyces*.

1 48. The method according to claim 45, wherein the host cell is a mammalian cell.

1 49. The method according to claim 45, wherein said CFTR polypeptide is a
2 mammalian CFTR polypeptide.

1 50. The method according to claim 45, wherein said CFTR polypeptide comprises
2 amino acid residue 351 through 650 of the human CFTR protein sequence.

1 51. The method according to claim 45, wherein said detectable gene is selected
2 from the group consisting of lacZ, *LEU2* and *HIS3*.

1 52. The method according to claim 45, wherein said DNA binding domain
2 comprises the DNA binding domain of GAL4 protein.

1 53. The method according to claim 45, wherein said transcriptional activation
2 domain comprises the transcriptional activation domain of GAL4 protein.

1 54. The method according to claim 45, wherein said CFTR polypeptides are
2 mutant CFTR polypeptides.

1 55. The method according to claim 45, wherein said CFTR polypeptide comprises
2 a mutation in the first nucleotide binding domain (NBD1).

1 56. The method according to claim 54, wherein said mutant CFTR polypeptide
2 contains a $\Delta F508$ mutation.

1 57. The method according to claim 45, wherein said CFTR polypeptide is a wild
2 type CFTR polypeptide.

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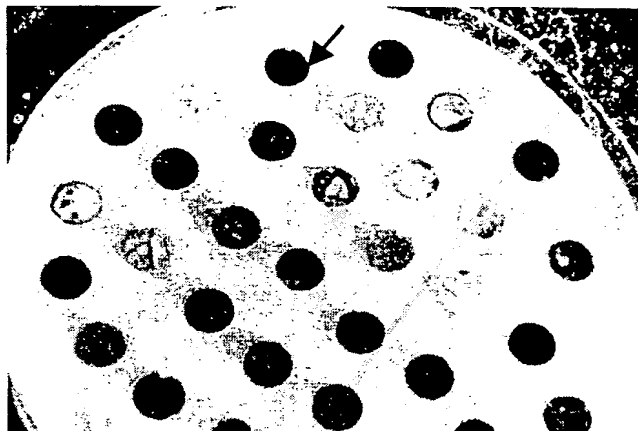


FIG. 1

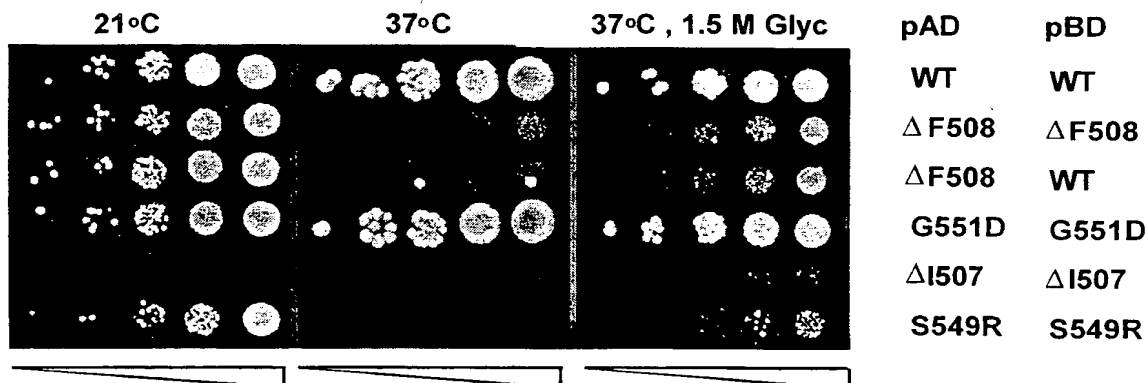


FIG. 2A

FIG. 2B

FIG. 2C

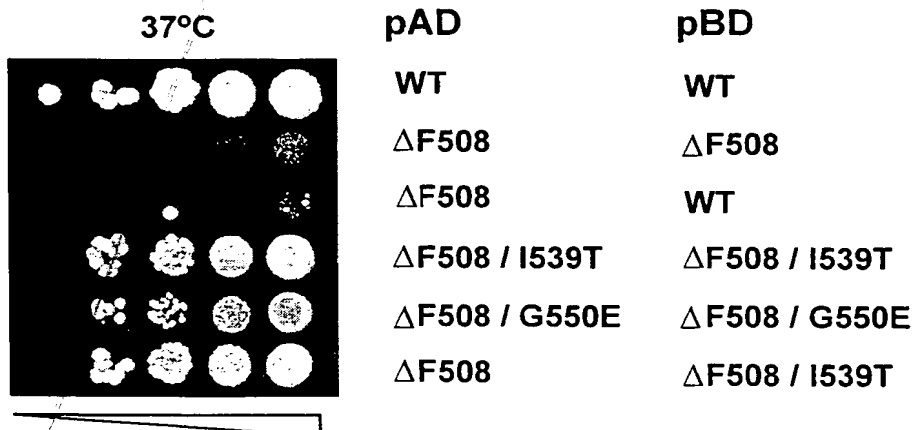


FIG. 3